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Nucleotide Sequence Analysis of Human T-Cell Lymphotropic Virus Type I pX and LTR Regions From Patients With Sicca Syndrome

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Human T-cell lymphotropic virus type I (HTLV-I) is associated with adult T-cell leukemia (ATL) and tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). Other inflammatory disorders may occur in HTLV-I-infected patients, such as sicca syndrome resembling Sjögren's syndrome. The sicca syndrome may be the unique clinical manifestation of HTLV-I infection, but is associated frequently with TSP/HAM, which could suggest that sicca syndrome might be an early event in disease progression to TSP/HAM in some cases. We investigated whether peculiar pX and LTR mutations could be related to sicca syndrome, or might argue the existence of clinical progression to TSP/HAM. pX, especially pX^{I}, pX^{II}, and pX^{IV} ORFs corresponding to Tax cytotoxic T-lymphocyte epitopes, and LTR regions from Caribbean patients who have sicca syndrome with or without TSP/HAM, ATL patients, and healthy carriers were sequenced. The sequences were aligned and compared with ATK-1 prototype and published sequences. LTR sequences exhibited 1.5–2.4% of divergence with ATK-1. pX-sequenced regions showed a lower homology within $p12^{I}$ encoding sequences. Only few mutations were found within functionally important regions, but were not associated specifically with the clinical status. Finally, no mutations that could be related to sicca syndrome or argue the existence of clinical progression to TSP/HAM were found. It would be of interest to study the clinical evolution of HTLV-I–sicca syndrome in patients and to determine HTLV-I sequences from peripheral blood and salivary glands at different stages. ***J. Med. Virol. 59:245–255, 1999.***

KEY WORDS: HTLV-I pathogenesis; DNA sequence; Sjögren's syndrome; TSP/HAM; $p12^{I}$; Caribbean basin

INTRODUCTION

Human T-cell lymphotropic virus type I (HTLV-I) is a human retrovirus associated etiologically with adult T-cell leukemia (ATL) [Poiesz et al., 1980; Hinuma et al., 1982] and a chronic neurodegenerative disease called tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) [Gessain et al., 1985; Osame et al., 1986]. Other diseases or clinical immunoregulatory disorders have been described in HTLV-I-infected patients, such as polymyositis [Francis and Hughes, 1989], arthritis [Nishioka et al., 1989], T-lymphocyte alveolitis [Sugimoto et al., 1987], infectious dermatitis [LaGrenade et al., 1990], uveitis [Ohba et al., 1989; Mochizuki et al., 1992], and sicca syndrome resembling Sjögren's syndrome [Vernant et al., 1988].

Sjögren's syndrome is a chronic exocrinopathy of presumed autoimmune etiology causing keratoconjunctivitis sicca and xerostomia, with a sialadenitis characterized by lymphocytic infiltration of the salivary and lachrymal glands [Wu and Fox, 1994]. To date, the pathogenesis of this syndrome remains to be clarified. Many studies strongly suggest that HTLV-I is involved in the pathogenesis of the disease in a subset of patients with Sjögren's syndrome in endemic areas, such as Japan [Eguchi et al., 1992; Terada et al., 1994; Na-

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kamura et al., 1997] and the Caribbean basin [Vernant et al., 1988; Hajjar et al., 1995]. The sicca syndrome observed in HTLV-I infected patients did not always fulfill all criteria for the diagnosis of primary Sjögren's syndrome, which vary according to the investigators, and it was frequently associated with neurological symptoms or disease such as TSP/HAM [Vernant et al., 1988; Hajjar et al., 1995; Nakamura et al., 1997]. Hajjar et al. [1995] found a sicca syndrome in almost 80% of HTLV-I-infected patients from Guadeloupe, French West Indies, half of whom had neurological disorders. However, in some cases, sicca syndrome has been reported to be the unique clinical manifestation of HTLV-I infection. Thus, it might represent an early event in disease progression to TSP/HAM outcome.

Tax, a protein encoded by the HTLV-I-pX regulatory region located between the *env* gene and the 3′ long terminal repeat (LTR), is a major transactivator of viral and cellular genes expression, and is involved in the pathogenesis of ATL and TSP/HAM [Franchini, 1995; Levin and Jacobson, 1997]. It has also been suggested that Tax might be involved in the pathogenesis of Sjögren's syndrome or related sicca syndrome. Thus, LTR-*tax*-transgenic mice developed an exocrinopathy resembling Sjögren's syndrome, the clinical severity of which correlated with the level of Tax expression in salivary epithelium [Green et al., 1989]. In addition, Tax is the dominant target of the CD8+ cytotoxic T-lymphocyte (CTL) response in TSP/HAM patients and in some HTLV-I-infected patients with Sjögren's syndrome [Kannagi et al., 1992; Pique et al., 1996]. HTLV-I-specific CTL response is suspected of playing a role in TSP/HAM pathogenesis and possibly in autoimmune-like disorders associated with HTLV-I infection, like sicca syndrome [Levin and Jacobson, 1997]. Furthermore, Niewiesk et al. [1995] showed that naturally occurring variants of Tax could impair its recognition by CTL and its transactivation function. Thus, it would be of interest to study the sequence of Tax CTL epitopes in isolates from HTLV-I infected patients with sicca syndrome and/or TSP/HAM, to detect subtle changes that could affect the CTL response.

Besides pX^{IV} open reading frame (ORF) encoding Tax, the pX region contains pX^{I} and pX^{II} ORFs potentially encoding at least three proteins, termed $p12^{I}$, $p30^{II}$, and $p13^{II}$, the functions of which remain unclear [Berneman et al., 1992; Ciminale et al., 1992]. Although pX^{I} and pX^{II} ORFs seem to be dispensable for virus replication or immortalization of primary lymphocytes in vitro [Derse et al., 1997; Robek et al., 1998], $p12^{I}$ has been recently shown to play an essential role in establishing persistent viral infection in vivo [Collins et al., 1998].

Previous molecular analyses of HTLV-I sequences from patients with ATL or TSP/HAM and from healthy carriers have shown low genetic variability between isolates, and have not revealed so far any disease-specific viral sequences [Daenke et al., 1990; Komurian et al., 1991; Saito et al., 1995]. However, some mutations have been linked to geographic origin, identifying three well-supported clusters: cosmopolitan, central African and Melanesian [Liu et al., 1996].

Recently, Gobinet-Georges et al. [1998] reported no specific mutation related to sicca syndrome in HTLV-I surface envelope glycoprotein gp46. In our study, we investigated whether sequence variants of pX and LTR regions could be detected in patients with sicca syndrome, TSP/HAM, and both TSP/HAM and sicca syndrome, which might argue the existence of clinical progression from sicca syndrome to TSP/HAM. Thus, we analyzed pX, especially pX^{I}, pX^{II}, and pX^{IV} ORFs corresponding to Tax CTL epitopes, and LTR regions of HTLV-I provirus from patients who have sicca syndrome with or without TSP/HAM, patients with ATL, and healthy carriers.

MATERIALS AND METHODS

Patients

The study comprised HTLV-I Caribbean or Guianese infected subjects, including 2 with sicca syndrome only, 4 with TSP/HAM and sicca syndrome, 1 with TSP/HAM only, 4 with ATL, and 4 healthy carriers. Most were Creoles, who are of mixed African and European descent, and one belonged to the Boni ethnic subgroup, consisting of Guianese people of African origin. The serological status was determined using an ELISA as a screening test (Cobas-Core, Roche, France). This was followed by Western blot analysis (Western blot 2.4, Genelabs Diagnostics, Singapore) as a confirmatory test. Sicca syndrome was diagnosed by the presence of objective evidence of lachrymal or salivary gland involvement, and confirmed by Schirmer's test, Jones' test, break-up time test, rose bengal or fluorescein tests on conjunctiva and cornea, and pathological histology of conjunctiva prints and labial minor salivary glands. Subjects with human immunodeficiency virus or hepatitis C virus infection, other disease processes and treatments that could result in sicca syndrome, were excluded. Table I summarizes the characteristics of the HTLV-I-infected patients.

Polymerase Chain Reaction

DNA was prepared from whole blood (Kontron Genomix kit, Montigny Le Bretonneux, France). Three pairs of oligonucleotides in the pX region and one pair in the LTR region of the HTLV-I genome were selected for use as primers [Chadburn et al., 1991; Komurian et al., 1991]. The characteristics of the primers used (Eurogentec, Seraing, Belgium) are shown in Table II. The PCR mixture contained 0.5–1 μg of DNA, 0.5 μM each of primers, 200 μM each of 4 dNTPs, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 2.5 units of *Taq* polymerase (Eurogentec). Samples were overlaid with 100 μl of mineral oil (Sigma, St. Louis, MO) and amplified in a DNA thermal cycler (Perkin Elmer Cetus, Foster City, CA). The PCR procedure involved an initial denaturation step at 94°C for 5 minutes. Amplifications of the pX region were performed through 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute. The cycling conditions for LTR am-

TABLE I. Characteristics of HTLV-I Infected Patients

Clinical status	Patient no.	Sex (M, F)	Age (years)	Ethnic origin	Geographic origin
Sicca syndrome	GUA1	M	68	Creole	Guadeloupe
	GUA2	F	48	Creole	Guadeloupe
TSP/HAM + sicca syndrome	GUA3	F	69	Creole	Guadeloupe
	GUA4	F	85	Creole	Guadeloupe
	GUA5	F	59	Creole	Guadeloupe
	GUA6	F	67	Creole	Guadeloupe
TSP/HAM	GUA7	F	75	Creole	Guadeloupe
Adult T-cell leukemia	FGU1	F	71	Creole	French Guiana
	FGU2	M	45	Creole	French Guiana
	FGU3	F	39	Creole	French Guiana
	FGU4	M	26	Boni	French Guiana
Healthy carrier	FGU5	F	70	Creole	French Guiana
	FGU6	F	?	Creole	French Guiana
	FGU8	F	?	Creole	Guadeloupe
	FGU9	M	52	Creole	Guadeloupe

M, male; F, female; TSP/HAM, tropical spastic paraparesis/HTLV-I associated myelopathy.

TABLE II. Nucleotide Sequences of Synthetic Oligonucleotide Primers

Designation	Sequence (5′–3′)	Region	Position[a]
P12A	TTCCAACTGTCTAGTATAGCCATC	pX	6774–6797
P12B	GAAGCTGTGCTTGACGGTTTGC	pX	7139–7160
SK43[b]	CGGATACCCAGTCTACGTGT	pX	7658–7669
SK44[b]	CTCGGCTATTGCGCAGGTAGC	pX	7496–7516
P40A	CCGCCAATCACTCATACAACC	pX	7571–7591
P40B	GGGCAAACAGTCTTCGGGTAG	pX	7919–7939
LTR1[b]	ACCATGAGCCCCAAATATCCCCC	LTR	9–31
LTR2[b]	TTAAAGAGAGGACTCTCACGATATC	LTR	722–746

[a]The locations were numbered according to the Japanese ATK-1 prototype sequence starting from the first LTR nucleotide [Seiki et al., 1983].
[b]These primers were previously described [Chadburn et al., 1991; Komurian-Pradel et al., 1991].

plification were as follows: 94°C for 2 minutes, 55°C for 1 minute and 72°C for 2 minutes for 30 cycles [Komurian et al., 1991]. The last extension time was increased to 5 minutes before cooling at 10°C. All the genomic DNAs were manipulated in a room free from amplified products. The recombinant plasmid pMT2 and DNA from uninfected peripheral blood mononuclear cells were used, respectively, as positive and negative controls in every PCR experiment. The PCR products were visualized by agarose gel electrophoresis with ethidium bromide staining.

Sequencing of PCR Products

The PCR products were purified using the QIAquick PCR purification kit (Qiagen, Courtaboeuf, France). Sequencing of both strands was carried out using the ABI PRISM Big Dye Terminator cycle sequencing kit (Perkin Elmer Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The nucleotide sequences were determined by using an automated DNA sequencer (Perkin Elmer Applied Biosystems). Sequence ambiguities were resolved by manual sequencing using the Sequenase version 2.0 DNA sequencing kit (U.S. Biochemical Corp., Cleveland, OH) according to the manufacturer's instructions. Two PCR products obtained from two independent PCR experiments were sequenced to confirm mutations, which have not been described previously.

Sequence Analysis

Sequence analysis was undertaken with PC/Gene software (IntelliGenetics, Mountain View, CA). Nucleotide and amino acid sequences were compared with the cosmopolitan HTLV-I sequence ATK-1 [Seiki et al., 1983] using NALIGN and PALIGN programs [Myers and Miller, 1988]. The CLUSTAL V program [Higgins et al., 1992] was used to perform multiple sequences alignments with the following published sequences (EMBL/Genbank accession numbers): CR1 (K02722), BOI (L36905), TSP1 (L76026), CH (M69044), XAV, BOU, AKR, SIE [Komurian et al., 1991], HS35 (D00294), YS (U19949), and H5 (M37299, M37301, M37303), which belong to the cosmopolitan cluster, EL (S74562) and MOMS (X83118), which belong to the Central African cluster, MEL5 (L02534), which belongs to the Melanesian cluster, and LAF and CHE, of unknown origin [Franchini, 1995].

RESULTS

LTR Sequence Analysis

Almost the entire LTRs from all DNA samples were sequenced. The nucleotide changes are summarized in Figure 1. Compared with ATK-1 as reference, LTR se-

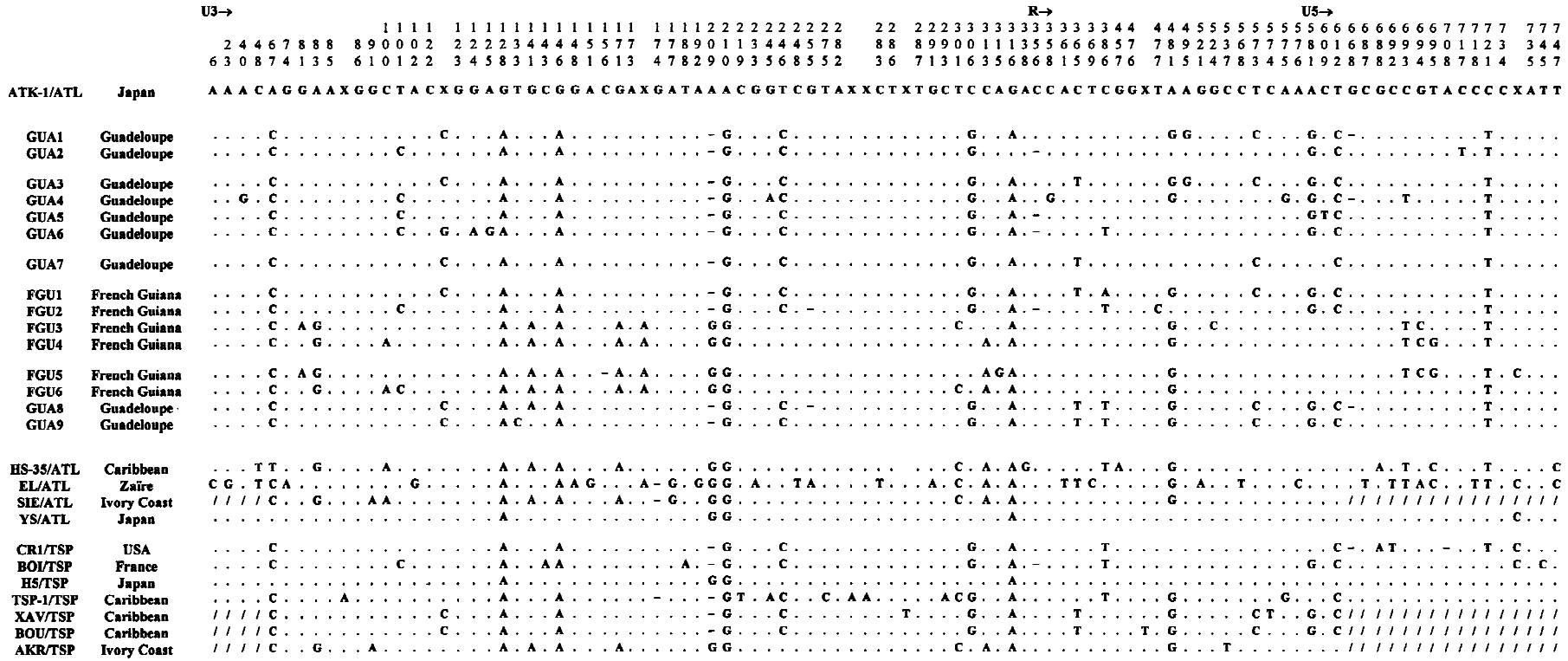

Fig. 1. Alignment of the nucleotide sequence of the LTR from different HTLV-I isolates. Only those changes observed with ATK-1 sequence are mentioned, and nucleotide positions are indicated vertically. Nucleotide positions are numbered according to the ATK-1 prototype sequence starting from the first LTR nucleotide [Seiki et al., 1983]. Successive points and dashes represent homologies and deletions, respectively. Insertions correspond to × in ATK-1 reference sequence. Slashes correspond to indetermined sequence. Our isolate identifications and clinical status are described in Table I. Identifications, clinical status, and geographic origin of other reference isolates are mentioned in the left side of the figure.

TABLE III. Mean of Nucleotide Divergence in Percentage Between ATK-1 and the Isolates Studied

	LTR			pX		
Clinical Status	U3	R	U5	nt 7496–7160	nt 7496–7669	nt 7571–7939
Sicca syndrome	2.2 (2.0–2.3)	0.5 (0–0.9)	2.1 (1.8–2.4)	2.0 (1.8–2.2)	0	0.6 (0.3–0.8)
TSP/HAM + sicca syndrome	2.5 (2.3–3.0)	1.0 (0–1.8)	2.1 (1.8–2.4)	1.7 (1.5–1.8)	0	0.6 (0.3–0.8)
TSP/HAM	2.3	1.3	1.8	1.8	0	0.3
ALT	2.8 (2.3–3.2)	1.0 (0.4–1.8)	1.5 (1.2–1.8)	2.2 (1.8–2.6)	0.2 (0–0.6)	0.7 (0.3–1.4)
Healthy carrier	3.0 (2.6–3.5)	0.9 (0.4–1.3)	1.4 (0.6–1.8)	2.2 (1.8–2.6)	0	0.6 (0.3–1.1)

The numbers in parentheses indicate the range in percentage of nucleotide divergence. The locations of pX amplified fragments were numbered according to the Japanese ATK-1 prototype sequence starting from the first LTR nucleotide [Seiki et al., 1983].

quences showed an overall variability from 1.5% to 2.4%, but exhibited a lesser divergence between them (0.1–2.3%). The regulatory region U3 showed the highest sequence variation, whereas the R region, which forms a rigid stem–loop RNA secondary structure implicated in Rex response and polyadenylation, was the most highly conserved (Table III). The TATA box, the polyadenylation signal, the splicing donor site, the three 21-bp Tax-responsive elements (TRE-1), which mediate Tax transactivation of the HTLV-I LTR, the Rex-binding element and the proximal stem of the R region were strongly conserved. However, some nucleotide changes occurred within functional regions, but none was linked to a disease status. Thus, two deletions were found within the TRE-1s in positions 156 and 255 in an ATL and two healthy carrier isolates, occurring in a GC-rich sequence essential for Tax transactivation [Paca-Uccaralertkun et al., 1994]. In addition, two substitutions (T^{573} to C and A^{575} to G) were observed within a suppressive sequence of the R region [Xu et al., 1996]. Some mutations were linked to the geographic origin (e.g., A^{83} to G), others were common to all isolates, suggesting the ATK-1 sequence is unusual in these locations (e.g., A^{210} to G). We carried out a comparative alignment analysis of our isolates with published LTR sequences (Fig. 1). As expected, the phylogenetic analysis showed that the 15 LTR sequences segregate into the cosmopolitan HTLV-I cluster (data not shown). However, most of the French Guianese isolates (FGU3, FGU4, FGU5, FGU6) were related to the West African subtype, which has been suggested to form a subgroup within the well-supported cosmopolitan cluster [Miura et al., 1994].

pX Sequence Analysis

Three fragments of pX region including overlapping parts of pX^{I} and pX^{II} ORFs (nt 6774–7160), and overlapping parts of pX^{II}, pX^{III}, and pX^{IV} ORFs (nt 7336–7494 and nt 7571–7939) were amplified and sequenced from all DNA samples. These PCR products corresponded to the entire $p12^{I}$ protein, and to different fragments including Tax [amino acid (aa) 14–65 and aa 92–214], the posttranscriptional regulator of viral gene expression termed as Rex (aa 33–84 and aa 111–189), $p30^{II}$ (aa 2–111 and aa 170–222) and $p13^{II}$ (aa 17–69).

The partial Tax and Rex encoding nucleic acid sequences were highly conserved, particularly within the SK43/SK44 amplified fragment (Table III). No insertion, deletion, or frameshift were detected in the sequenced regions. The three pX-sequenced regions of some isolates (GUA1, GUA3, GUA7, GUA8, FGU1) were entirely similar independently from clinical status (data not shown). Thus, the nucleotide sequences from patients with TSP/HAM–sicca syndrome did not share more or particular mutations compared to those from patients with sicca syndrome or TSP/HAM only.

Most of the substitutions resulted in aa changes. However, the partial Tax fragments had a particularly high conservation within the amino terminus, which contains the nuclear localization signal, the zinc finger-like domain and a major immunodominant T-cell epitope (Fig. 2). Three Tax mutations were found in other functional regions, located within a linear immunodominant B-cell epitope [Lal, 1996] and an overlapping potential HLA-A2-restricted T-cell epitope [Parker et al., 1994]. The Rex protein showed more aa changes, but only two substitutions (A^{58} to V, and H^{121} to R) were found within domains that have a known or supposed functional importance (Fig. 3).

Most of the nucleotide changes found in the pX^{I} and pX^{II} ORFs sequenced regions resulted in aa substitution in $p12^{I}$ and/or $p30^{II}$ proteins. Although $p12^{I}$ aa sequences exhibited the highest divergence compared with ATK-1 (4–6.1%), they were very closely related to one another (0–5%) (Fig. 4). Thus, $p12^{I}$ was identical in several isolates from patients with different clinical status or from healthy carriers (e.g. FGU2, GUA5 and GUA6). Most of the changes occurred in the putative transmembrane domains encompassing aa 12–32 and aa 48–68, but did not modify the highly hydrophobicity of the transmembrane regions (75.4–76.5% of hydrophobic aa). Some aa (P^{23}, S^{25}, and G^{51}) were only observed within the ATK-1 and YS isolates, and might be specifically linked to their Japanese origin. Other mutations were related to the subtype C according to Miura et al. [1994] (e.g., D^{26} to E). One coding change (Q^{47} to L), not described previously to our knowledge, was observed in a sicca syndrome isolate (GUA2) within the 36 to 48 aa proline-rich domain, which is important for the binding to the 16-kDa subunit of the cellular vacuolar H^{+}–ATPase proton pump. An error made by *Taq* polymerase was ruled out by sequencing another PCR product. Other domains were tightly conserved throughout the different isolates, particularly the leucine-rich domains.

Some mutations in $p30^{II}$ and $p13^{II}$ were also similar

		← a →← b →																	
									1	1	1	1	1	1	1	1	1		
		2	5		5	9	9	0	0	1	1	2	7	7	9	9			
		1	5		6	4	6	5	7	2	9	0	4	7	1	3			
ATK-1/ATL	Japan	G	T	X	W	I	H	L	Q	Y	Y	M	C	G	I	E			
GUA1	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
GUA2	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
GUA3	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
GUA4	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
GUA5	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
GUA6	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
GUA7	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
FGU1	French Guiana	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
FGU2	French Guiana	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
FGU3	French Guiana	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
FGU4	French Guiana	.	.		.	.	.	.	R	H	.	.	.	.	.	.			
FGU5	French Guiana	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
FGU6	French Guiana	.	.		.	.	.	.	.	.	.	K	.	.	.	.			
GUA8	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
GUA9	Guadeloupe	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
HS-35/ATL	Caribbean	.	.		.	.	.	.	.	.	.	.	.	.	.	K			
EL/ATL	Zaïre	.	.		.	.	.	.	.	.	.	.	.	D	M	.			
SIE/ATL	Ivory Coast	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
YS/ATL	Japan	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
BOI/TSP	France	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
H5/TSP	Japan	R	.		.	.	.	.	.	.	.	.	.	.	.	.			
TSP-1/TSP	Caribbean	.	.		.	.	.	.	.	.	.	.	.	.	.	K			
XAV/TSP	Caribbean	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
BOU/TSP	Caribbean	.	.		.	.	.	.	.	.	.	.	.	.	.	.			
AKR/TSP	Ivory Coast	.	.		.	.	.	.	.	.	C	.	.	.	.	.			
MEL-5/HC	Solomon Islands	.	.	W	*	T	.	F	.	.	.	.	.	.	.	.			
CH/HC	Caribbean	.	.		.	.	.	.	.	.	.	.	D	.	.	.			
MOMS/HC	Zaïre	.	.		.	T	D	.	.	.	.	.	.	S	M	.			

Fig. 2. Deduced amino acid alignment of Tax (aa 14–65 and aa 92–214) from different HTLV-I isolates. The single letter amino acid code is used. Only those changes observed with ATK-1 sequence are mentioned, and amino acid positions are indicated vertically. Points represent homologies, and insertions correspond to × in ATK-1 reference sequence. Termination codons are represented by *. "a" and "b" indicate amino acid changes within aa 14–65 and aa 92–214 regions of Tax, respectively.

in several isolates (Fig. 5). The nucleotide change A^{6973} to T observed in a sicca syndrome isolate (GUA2) resulted in a premature translational termination codon, potentially producing $p13^{II}$ and $p30^{II}$ truncated proteins.

DISCUSSION

Previous studies have failed to detect mutations linked to ATL, TSP/HAM, or asymptomatic carrier status [Daenke et al., 1990; Komurian et al., 1991; Ratner et al., 1991]. However, the other clinical or subclinical disorders related to HTLV-I infection have been rarely considered. In this study, HTLV-I pX and LTR regions from HTLV-I-infected subjects with sicca syndrome, TSP/HAM and sicca syndrome, ATL and healthy carriers were examined. The aim was to determine whether sequence variants could be linked to sicca syndrome and/or could argue a hypothetical evolution from sicca syndrome to TSP/HAM. Nucleotide and amino acid sequences were compared with one another and with already published sequences from infected patients of various geographic origin and HTLV-I-associated disease.

As described previously [Komurian et al., 1991; Ratner et al., 1991], the region U3 was the most divergent region of the LTR, whereas R was the most highly conserved. The mutations found did not correlate with a particular clinical status, but might have functional effects. Thus, two substitutions were observed within a suppressive sequence of the R region, which binds ATF-2 and CREB factors. Xu et al. [1996] showed that single-base substitutions occurring naturally within this segment (nt 572–579) resulted in reducing protein binding, and were associated with derepression of the LTR promoter when compared with the wild-type LTR promoter. Xu et al. (1996) reported an apparent higher incidence of these mutations among TSP/HAM patients. In our study, only three TSP/HAM-sicca syndrome or TSP/HAM isolates exhibited a mutation within this segment. In addition, minor differences in LTR sequence can affect its activity according to the cell type used, although no evidence of a link between promoter activity and TSP/HAM or ATL disease outcome has been clearly demonstrated [Gonzalez-Dunia et al., 1993]. It would be of interest to test an activity of LTR from isolates with sicca syndrome, TSP/HAM and sicca syndrome, or TSP/HAM in salivary cells lines.

High-sequence conservation of the amino-terminal region and midregion of the Tax protein was found in all isolates. The main Tax CTL epitope is located in the N-terminus, but other CTL epitopes have been reported in the middle region of the protein, which also contains a B-cell epitope [Kannagi et al., 1992; Parker et al., 1994; Niewiesk et al., 1995; Lal, 1996]. It has been demonstrated that naturally occurring sequence variations in CTL epitope-coding region of Tax could impair its recognition by CTL and its transactivation function [Niewiesk et al., 1995]. In addition, Tax has been shown to be more variable between and within healthy carriers than patients with TSP/HAM, suggesting a stronger selection for amino acid conservation in the latter [Niewiesk et al., 1994]. One could expect to find differences between sicca syndrome and TSP/HAM patients. However, no mutation within sequenced CTL epitopes in sicca syndrome, TSP/HAM-sicca syndrome and TSP/HAM isolates were detected. Only three mutations were observed within a potential B-cell epitope and an overlapping CTL epitope, two in an ATL isolate, and one in a healthy carrier. The possibility that these mutations might impair the immune responsiveness to HTLV-I cannot be excluded.

Compared to other HTLV-I genomic regions, $p12^{I}$-encoding sequences have been investigated to a lesser extent. In our study, $p12^{I}$ was entirely identical in several isolates from patients with different HTLV-I-associated clinical status (e.g., samples FGU2, GUA5, and GUA6), and no deletion was detected in any of the isolates. Deletions in pX^{I} ORF appear to be a rare event [Ratner et al., 1985]. The amino acid sequences of $p12^{I}$ have been reported to be conserved, particularly in some repetitive motifs proposed to be putative SH3 binding domains [Franchini, 1995] or leucine-zipper motifs [Saksena et al., 1997]. The mRNAs encoding

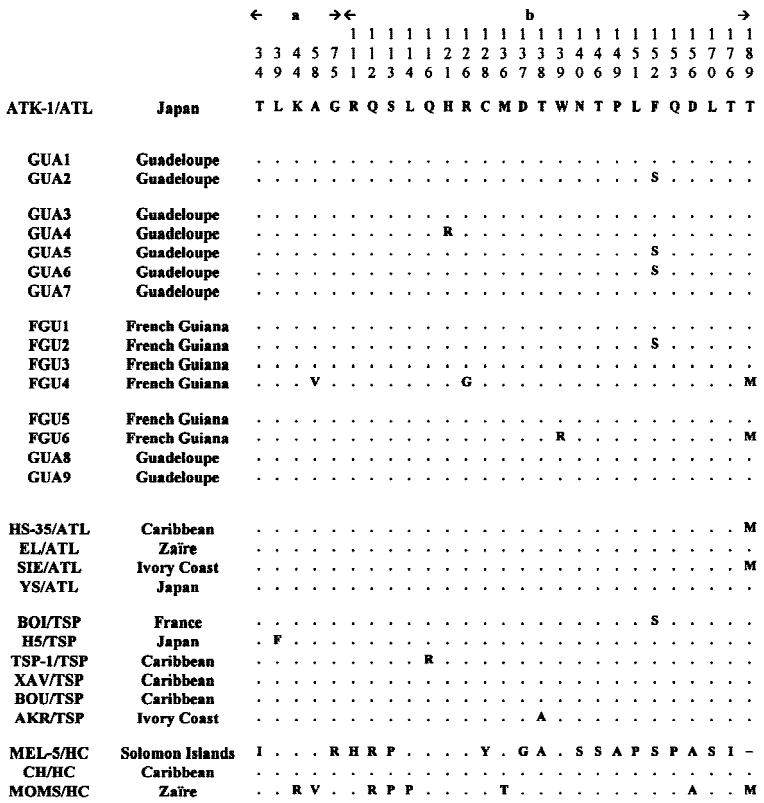

Fig. 3. Deduced amino acid alignment of Rex (aa 33–84 and aa 111–189) from different HTLV-I isolates. The single letter amino acid code is used. Only those changes observed with ATK-1 sequence are mentioned, and amino acid positions are indicated vertically. Successive points and dashes represent homologies and deletions, respectively. "a" and "b" indicate amino acid changes within aa 33–84 and aa 111–189 of Rex, respectively.

$p12^{I}$, $p30^{II}$, and $p13^{II}$ have been detected in infected cells lines, fresh lymphocytes from infected patients, and cells transfected with HTLV-I provirus clones. However, the corresponding proteins have not yet been found in infected cells. $p12^{I}$ is a putative endomembrane-associated protein, and cooperates with the related bovine papillomavirus E5 protein in the transformation of mouse cells and the binding to a vacuolar proton pump, suggesting a potential role in leukemogenesis [Franchini et al., 1993]. $p12^{I}$ also binds to the β and γ chains of the interleukin-2 receptor in vitro, potentially impairing the receptor signaling [Mulloy et al., 1996]. Most of the amino acid changes detected in our study occurred in the putative transmembrane domains, but the hydrophobicity of these regions was not impaired. Whether these mutations could have a functional significance remains to be determined.

A premature stop codon was found within pX^{II} ORF region in a healthy carrier isolate (GUA2), which could result in truncated $p13^{II}$ and $p30^{II}$ proteins. Defective pX^{II} ORFs have been reported in the central nervous system of a patient with TSP/HAM [Kira et al., 1994], and in leukemic cell DNA [Berneman et al., 1992; Chou et al., 1995], but the biological significance of this finding is unknown. No premature stop codon was found in TSP/HAM and sicca syndrome isolates. However, no particular pattern of mutations was related to clinical status. $p30^{II}$, a nucleolar protein, exhibits a distant homology to the Oct and Pit families of transcription factors, but this function has not been demonstrated yet [Ciminale et al., 1992]. Recently [D'agostino et al., 1997], $p30^{II}$ has been suggested to have a functional role in the metabolism of cellular or viral mRNAs.

pX^{I} and pX^{II} ORFs have been shown to be dispensable for virus replication, for immortalization of primary lymphocytes, and would have no discernable effect on virus gene expression in vitro [Roithmann et al., 1994; Derse et al., 1997; Robeck et al., 1998]. However, a recent report showed that $p12^{I}$ could play an essential part in the establishment of persistent viral infection in vivo [Collins et al., 1998]. It is still unclear whether these proteins are ever produced at detectable levels in certain cell types or under specific conditions. These proteins could be expressed in the salivary glands, and could exert subtle effects on the infected cells in vivo in a cell-type- or in a tissue-type-specific manner.

Gobinet-Georges et al. [1998] reported no specific mutation related to sicca syndrome in HTLV-I glycoprotein gp46 encoding sequences. Likewise, direct comparisons of LTR and pX regions did not reveal obvious sequence relationships according to clinical status. No evidence of peculiar mutations was detected in samples from patients with sicca syndrome and argued the existence of clinical progression to TSP/HAM. It would be of interest to study the clinical evolution of patients

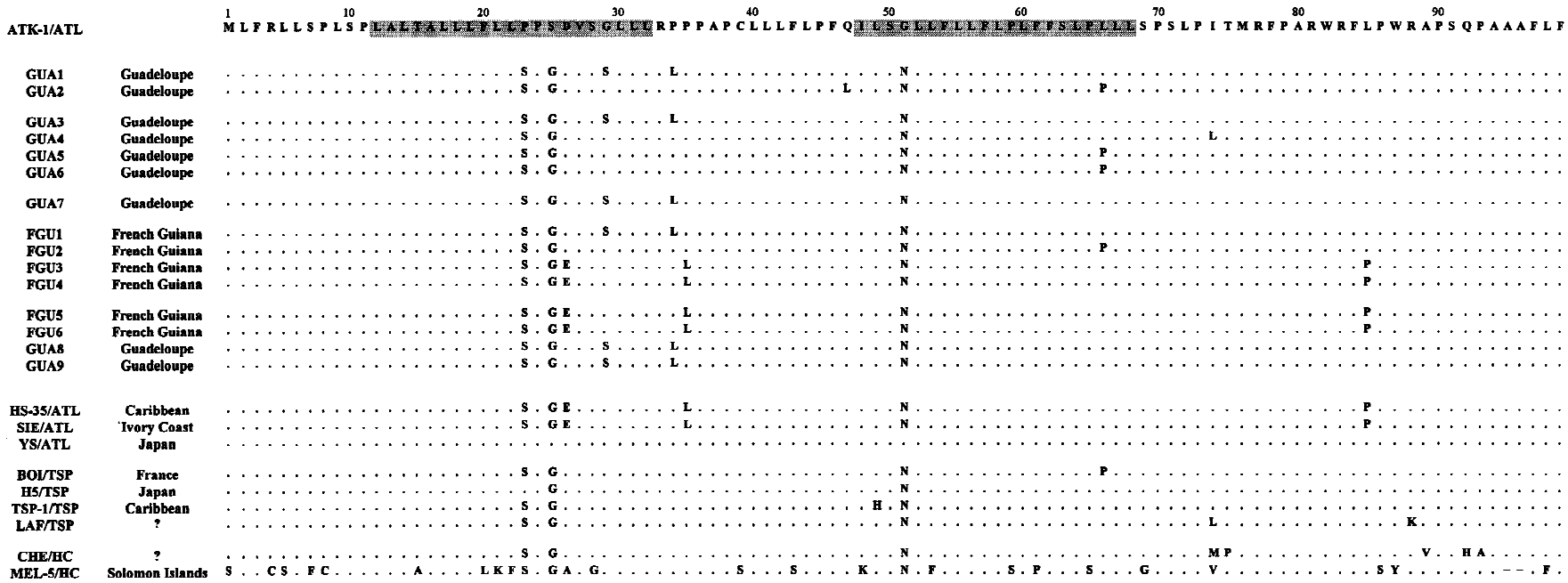

Fig. 4. Deduced amino acid alignment of p12^{I} from different HTLV-I isolates. Successive points and dashes represent homologies and deletions, respectively. The single letter amino acid code is used. Putative transmembrane domains are shaded.

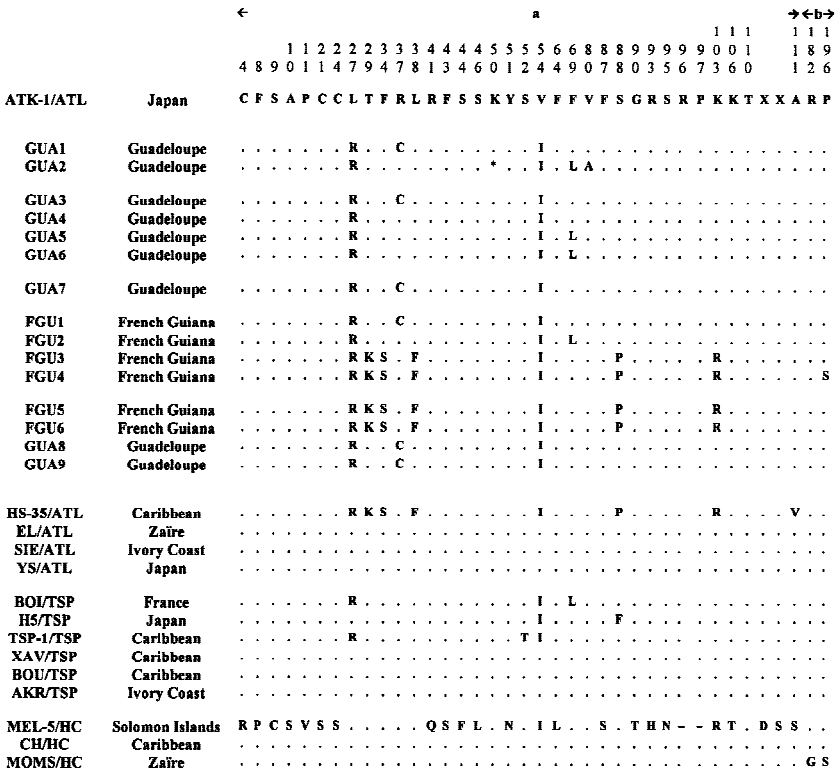

Fig. 5. Deduced amino acid alignment of $p30^{II}$ (aa 2–111 and aa 170–222) from different HTLV-I isolates. The single letter amino acid code is used. Only those changes observed with ATK-1 sequence are mentioned, and amino acid positions are indicated vertically. Successive points and dashes represent homologies and deletions, respectively. Insertions correspond to × in ATK-1 reference sequence and termination codons are represented by *. "a" and "b" indicate amino acid changes within aa 2–111 and aa 170–222 of $p30^{II}$, respectively.

with sicca syndrome and to determine HTLV-I sequences in peripheral blood and in salivary gland samples from patients with sicca syndrome at different clinical stages. In addition, it is not impossible that salivary gland infection is initiated by a limited number of variants or even one particular variant, which could be locally selected. The results obtained in *tax*-transgenic mice studies [Green et al., 1989] suggested that HTLV-I had salivary and lachrymal epithelial cell tropism, and Tax could play an important part in the sicca syndrome disease process. Furthermore, a better characterization of the productively infected cells in the salivary gland might provide new insights in the pathogenesis of HTLV-I-associated sicca syndrome.

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